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Research Article
Pro-oxidant Activity and Genotoxicity of the *Astronium fraxinifolium* Using Wing SMART and *Allium cepa* Test

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Abstract

Astronium fraxinifolium is an arboreal species found throughout the Brazilian Cerrado region and used in folk medicine as antimicrobial, anti-hemorrhagic and healing. Pro-oxidant activity of extracts of *A. fraxinifolium* through Relative Electrophoresis Mobility (REM) of Bovine Serum Albumin (BSA) protein in presence of the extract and Cu^{2+} and also the genotoxic potential through Somatic Mutation and Recombination Test (SMART) and *Allium cepa* tests has been investigated during this study. In the REM, the extracts acting exclusively on BSA resulted in a band formation with higher molecular weight than BSA, probably due to oxidative action of the extracts. In presence of Cu^{2+} and extracts occurred protein fragmentation due to Cu^{2+} oxidative action potentiated by the extracts. In SMART test the frequency of mutant spots increases with the increased concentration of extract *A. fraxinifolium* (50 mg mL^{-1} = 1.40 and 100 mg mL^{-1} = 2.66). The HB cross shows a decrease in the total of mutant spots frequency for the different treatments (50 mg mL^{-1} = 6.60 and 100 mg mL^{-1} = 3.25). Evaluation of *A. cepa* test demonstrated the following results (concentration extract = chromosomal abnormalities) 1 mg mL^{-1} = 85, 10 mg L^{-1} = 61 50 mg mL^{-1} = 53, 100 mg mL^{-1} =33 and MMS 10 mg L^{-1} = 50. Genotoxic and cytotoxic actions can be explained by the actions of tannins present in its composition. But there may be other substances that also act for such results. The genotoxicity in medicinal plants contributes to therapeutic safety warning that although the use of medicinal plants is an inexpensive and non-aggressive method it can cause harmful effects if used incorrectly.

Key words: Medicinal plants, micronucleus, tannins, mutagenic tests, oxidation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus Astronium (Anacardiaceae) is represented by arboreal species found in the neotropical region of Brazil (Santin, 1991; Lorenzi, 1992; Santos *et al*., 2007). Among the species of this genus, there is *Astronium fraxinifolium*, which has a stem rich in tannic substances (Leite, 2002). Its barks are used as decoction for the treatment of gastric and vaginal inflammation (Matos, 1989) and indicated by Cruz (1982) in the treatment of diarrhea and dysentery due to its astringent properties. Moreover, *A. fraxinifolium* is widely used in folk medicine as an antiseptic, antimicrobial, anti-hemorrhagic, healing, anti-inflammatory, antiulcerogenic besides treating fever, improving the development of children's teeth and for dermatological treatment (Leite, 2002; Macedo and Ferreira, 2004).

The Somatic Mutation And Recombination Test (SMART) was designed to detect loss of heterozygosity, phenotypically expressing the recessive genetic markers in wing's hair of *Drosophila melanogaster*. This bioassay is effective to quantify the potential of chemical and physical agents to cause recombination and genetic mutations (Graf *et al*., 1996; Santos *et al*., 1999; Vogel, 1980; Spano *et al*., 2001; Da Silva *et al*., 2008; Sinigaglia *et al*., 2004, 2006; Padua *et al*., 2013; Anter *et al*., 2014). For this purpose, two types of crossings are used: Standard cross (ST) (Graf *et al*., 1989) and high bio activation (HB) cross (Graf and van Schaik, 1992). Individuals from the ST cross have basal metabolism of cytochrome P450 (CYP450) and are used to detect direct actions of genotoxic agents. Those from HB cross are characterized by an increased metabolism of CYP450 enzymes and are used to detect the indirect action of genotoxic agents (Frolich and Wurgler, 1989, 1990; Graf and van Schaik, 1992; Saner *et al*., 1996).

Another test used for the evaluation of genotoxicity is the analysis of chromosomal aberrations, determination of the mitotic index and the frequency of micronuclei in meristematic cells of *Allium cepa* roots. This has been an efficient test to indicate the presence of mutagenic chemicals (Levan, 1938; Fiskesjo, 1985) due to its sensitivity and also has a good correlation with mammalian test systems (Chauhan and Gupta, 2005). Besides the environmental monitoring, the genotoxicity test of *A. cepa* has been applied in analysis of natural chemical compounds, mainly to check the safety and genotoxicity of plant extracts used in folk medicine (Camparoto *et al*., 2002; Knoll *et al*., 2006).

Due to the genotoxic and mutagenic effects of extracts, fractions and isolated compounds used in folk medicine, there is a need to investigate their genotoxic and mutagenic effects. For this, SMART and the test of *A. cepa* were used.

MATERIALS AND METHODS

Collection of plant material: The bark of *A. fraxinifolium* were collected from the urban area of Uberlândia/MG in September, 2009 (18°54'18,43''S and 48°13'56,13''W), in a sustainable manner and without causing environmental impact to specimens. A herbarium specimen (Accession No.198AF) was deposited in the herbarium of Instituto Florestal do Estado de São Paulo, Assis, Brazil.

Preparation of the *A. fraxinifolium* bark extract: The bark was dried in a forced air oven and crushed. Bark poweder was extracted (3x) with 70:30 hydroalcoholic solution (ethanol/water) at a proportion 1:10 of plant and solution (w/v) under mechanical stirring for 24 h. Extract was collected and taken to the rotary evaporator to remove the alcohol. The resulting extract was dried in a drying chamber to obtain dry extract.

Chemical composition: Phytochemical evaluation of the crude extract for total phenol and flavonoid content was performed by Thin Layer Chromatography (TLC) in triplicate.

For the calculation of equivalence with the standard gallic acid (total phenols) and quercetin (total flavonoids the following equations were used:

$$\text{Phenols} = \frac{(A - 0.21245)}{0.0697}$$

where, A is average absorbance,

$$\text{Flavonoids} = \frac{(B + 0.1835)}{0.01202}$$

where, B is average absorbance.

Evaluation of the pro-oxidant activity by Relative Electrophoresis Mobility (REM): The REM was adapted from Hsieh *et al*. (2005) and Toda (2005). Bovine Serum Albumin (BSA) 2 mg mL^{-1} was diluted in Phosphate Buffered Saline (PBS) (10 mM, pH 7.4) and incubated with Cu^{2+} (2 mM) at 37°C for 10 days in the presence or absence of the ethanolic and hydro ethanolic (70%) *Astronium fraxinifolium* bark extracts (200 µg mL^{-1}). Electrophoresis of BSA was performed using polyacrylamide gels (SDS-PAGE). It was prepared according to the standard technique (Encor Biotechnology Inc.). Running

gel solution was utilized in 12% of acrylamide and the stacking gel at 5%. Proteins were stained with 0.25% Coomassie Blue R-250. Results were expressed in the REM using that of native BSA as the base.

Somatic mutation and recombination test: The lineages of *Drosophila melanogaster* with modified wings are obtained from two crosses: Standard (ST) and High Bioactivation (HB). The ST cross was performed by mating between virgin females flr^3/In(3LR)TM3, ri p^p sep l(3)89Aa bx^{34e} e Bd^S and males mwh/mwh (Graf *et al*., 1984, 1989), while the High Bioactivation cross (HB) was performed by mating virgin females between ORR; flr^3/In(3LR)TM3, ri p^p sep l(3)89Aa bx^{34e} e Bd^S and males mwh/mwh. Marked trans-heterozygous (MH: mwh+/+flr^3) with phenotypically normal wings and balanced heterozygous (BH: mwh+/TM3, Bd^S) with phenotypically serrated wings were obtained in both crosses, (Graf and van Schaik, 1992). Eggs were collected from both crosses during a period of eight hours in vials containing a solid agar-agar base (3% agar in water) and a layer of *S. cerevisiae* supplemented with sucrose. After three days, when reaching the 3rd larval stage (72±4 h) they were washed with ultrapure water (Milli Q) and collected with the aid of a fine mesh sieve. For chronic treatment, the larvae were placed in glass vials containing 1.5 g of instant mashed potatoes (Yoki Alimentos S.A., Brazil) and 5 mL of solution with *Astronium fraxinifolium* extract in different concentrations (50 and 100mg mL^{-1}). Positive and negative controls used in the experiments were, respectively, the urethane (10 mM) and mineral water. The experiment was conducted under controlled temperature (25°C±2). Emerging adults of both crosses, carriers of both types of genotypes: mwh+/+flr^3 or mwh+/TM3, Bd^S were collected and preserved in 70% ethanol. The wings were extracted and embedded in Faure's solution (30 g of gum Arabic, 20 mL glycerol, 50 g of chloral hydrate and 50 mL of distilled water), expanded and mounted on glass slides with Etelan® (Merck) and analyzed with microscope at 40x magnification. The frequency and size of single or twin spots were noted. Single spots (mwh or flr^3) are the result of mutational events, chromosomal aberrations or mitotic recombination (crossover between two genetic markers). Twin spots (mwh or flr^3) are unique result from mitotic recombination (crossover between the marker flr^3 and the centromere of chromosome). The wings of BH cross are mounted and analyzed after verification of positive results obtained in the progeny MH. On the wings of BH flies only mwh single spots can be observed. These spots are due to mutational events only because recombination is suppressed in inversion-heterozygous cells with the multiply inverted TM3 balancer chromosome (Graf *et al*., 1984).

Statistical analyses: For the cross ST, 30 flies were analyzed for the concentration of 50 mg L^{-1} and 29 flies for concentration of 100 mg L^{-1}, while for the cross HB 20 flies were analyzed for both treatments. The procedure of multiple decisions was used to analyze the data (Frei and Wurgler, 1988) resulting in three different diagnoses: Negative, positive and inconclusive. The frequency of each type of spot (small single, large single and twin) and total frequency of spots per fly from each treatment were compared in pairs (Kastenbaum and Bowman, 1970) with p = 0.05 (Frei and Wurgler, 1988, 1995). All weak and inconclusive results were analyzed as nonparametric: Mann-Whitney and Wilcoxon U-test ($\alpha = \beta = 0.05$) (Frei and Wurgler, 1995). Based on the frequency of clones induction per 10^5 cells was calculated as follows:

$$\text{Frequency of mutation (FM)} = \frac{\text{Frequency of colones in flies BH}}{\text{Frequency of colones in flies MH}}$$

$$\text{Frequency of recombination (FR)} = 1\text{-Frequency of mutation (FM)}$$

$$\text{Total Frequency of Spots (FT)} = \frac{\text{Total spots observed in MH flies (considering spots mwh and flr}^3\text{)}}{\text{No. of flies}}$$

Statistical comparison of survival rates was performed by chi-square test because of the independent sample.

The data analyzes were conducted by Biostat 5.0 software. The total number of chromosomal aberrations and mitotic index were analyzed according to analysis of variance (ANOVA) with statistically different values for $p \leq 0.05$.

Genotoxicity of *Allium cepa*: Onion bulbs were obtained in local trade. Before starting the test the onions were peeled and dried roots were removed without damaging the root primordia. The basic protocol was followed according to Fiskesjo (1985, 1993). The bulbs were placed in beakers with their button primary roots submerged into culture solution (Hoagland's solution) (Beraud *et al*., 2007; Hoagland and Arnon, 1950) being changed every 24 h during 72 h. After this period, the Hoagland's solution was replaced by the following treatments: (1): Mineral water as negative control, (2): Methyl Methane Sulfonate (MMS) 10 mg L^{-1} as positive control and (3): Ethanollic extract of *A. fraxinifolium* diluted in mineral water in concentrations 1, 10, 50 and 100 mg mL^{-1}. After the treatment period, bulbs of *A. cepa* returned to beakers containing Hoagland's solution for 24 h. This part

of the experiment was conducted in BOD incubators with temperature (22±1°C) and (6/18 h light/dark) light controlled. After the end of exposure, the roots of the bulbs were removed and fixed in absolute ethanol: Glacial acetic acid (3:1, v/v). For mounting the slides, the roots of *A. cepa* were hydrolyzed in 1M HCl at 60°C for 8 min and then stained with a solution of 2% acetocarmine. The roots were then placed on a glass slide and fixed with 45% acetic acid. The slides were examined in an optical microscope at 100x magnification. One thousand cells were counted per slide, 5 slides per treatment and control. These were evaluated and quantified for Chromosomal Aberrations (CA), micronuclei (MN) and the Mitotic Index (MI) (Fiskesjo,1985; Fiskesjo *et al*., 1997):

$$\text{CA frequency } (\%) = \frac{\text{No.of aberrant cells}}{\text{Total quantified cells in each extract concentration}}$$

$$\text{Frequency MN} = \frac{\text{MN}}{\text{Cells observed were determined}}$$

(Bakare *et al*., 2000).

RESULTS

Chemical composition: The TLC was performed with mobile phases, (1): Dichloromethan, methanol (98:2, v:v) and (2): Butanol; acetic acid; distilled water (4:1:5, v:v:v). Before application of the telltale, was observed in the elution camera UV at wavelengths of 254 and 366 nm to reveal possible compounds with conjugated double bonds. The silica plates were stained with anisaldehyde, dragendorff, ferric chloride, polyethylene glycol (PEG-NP) (Table 1).

Evaluation of the pro-oxidant activity by Relative Electrophoresis Mobility (REM): In Fig. 1, there are formation of a new band in lane 1 and 2, above the BSA band, that is resulting of the action of extract hydroethalic and ethanolic on BSA, respectively. And in lane 3, with BSA only with copper, it is possible to see a change; a small band of lower molecular weight appeared. In the lanes 4 and 5, occurred fragmentation by Cu^{2+} oxidative action, potentiated by the extracts. In the lane 4, with hydroethanolic extract, the oxidative action was more intense because it is possible to see more fragments from the protein and the colour of BSA band is less intense on that specific run (Fig. 1).

Analysis of Somatic Mutation and Recombination Test (SMART): The test for detection of mutation and recombination in somatic cells of *D. melanogaster* wings two crossings are used, (1): Standard cross (ST) in which males "mwh" (mwh+/mwh+) are crossed with virgin females of strains flr^3 (+flr^3/TM3 Bd^5) and (2): high metabolic bioactivation cross (HB) in which males "mwh" (mwh+/mwh+)) are mated with females ORR; flr^3 (ORR/ORR;+flr^3/TM3 Bd^5). Both crosses produce trans-heterozygous individuals marked (mwh+/+flr^3) (MH) (Graf *et al*., 1984, 1989) (Fig. 2).

After preparing the treatments, the extract of the bark of *A. fraxinifolium* was assessed in *D. melanogaster* larvae in the third stage of development of ST and HB crosses, they are treated chronically over a period of approximately 48 h. The treatment was done in duplicate. The analyses of the experiment were made according to Frei and Wurgler (1988, 1995). After verifying statistical differences between the results of independent experiments, the data of ST and HB crosses are arranged in Table 2.

According to the results in Table 3, ST cross showed positive aspects in the twin spots (12) and total spots (42) for the concentration of 50 mg mL^{-1} of *A. fraxinifolium*. This same crossing with a concentration of 100 mg mL^{-1} of *A. fraxinifolium* showed positive results in all four criteria (small single spots, large single spots, twin and total spots). The HB cross showed that for concentrations of 50 and 100 mg mL^{-1} of *A. fraxinifolium*, the results are positive in all criteria examined.

Figure 3 shows the total frequency of spots for different types of treatment, negative control and positive control. For the ST cross, the frequency of mutant spots increases with the increased concentration of crude hydroalcoholic extract of

Table 1: Presence class of compounds of the crude hydroalcoholic bark extract of of *A. fraxinifolium* after elution of the TLC in the mobile phases 1 and 2, applying the revealing

	Revealing			
Mobile phases	Anisaldehyde	Dragendorrf	Ferric chloride	NP-PEG
1-Dichloromethane:methanol	-	-	-	-
2-Butanol:acetic acid:distilled water	-	-	+	±

-: Reaction negative to compounds class, +: Reaction positive to compound class, ±: Reaction inconclusive

bark of *A. fraxinifolium* (50 = 1.40 and 100 = 2.66 mg mL^{-1}). The HB cross shows a decrease in the total of mutant spots frequency for the different treatments (50 = 6.60 and 100 mg mL^{-1} = 3.25).

Analysis genotoxicity test of *Allium cepa*: The *Allium cepa* test was done with different concentrations (1, 10, 50 and 100 mg mL^{-1}) of hydroalcoholic bark extract. It was observed that a significant decrease in the MI to the concentration of 100 mg mL^{-1} (2.92±6.16) compared to other treatments (1 = 6.44±10.56, 10 = 5.72±11.56 and 50 = 5.4±11.97 mg mL^{-1}) and controls (distilled water = 6.82±14.43 and MMS = 5.4±11.83).

A significant increase of chromosomal aberrations was observed with increasing concentrations of 1 mg mL^{-1} (85), 10 mg mL^{-1} (61) and 50 mg mL^{-1} (53) compared to controls (distilled water = 3 and MMS = 50). Regarding the

Table 2: Total phenols and flavonoids of hydroethanolic bark extract of *A. fraxinifolium*

Extracts (µg mL^{-1})	Total phenols[a]	Total flavonoids[b]
100	-	-
250	3.41	1.61
500	7.15	1.75
1000	7.74	1.92
3000	7.88	3.35

[a]mg gallic acid equivalent per gram of extract, [b]mg of quercetin equivalen per gram of extract

Table 3: Frequency of mutant spots observed in the offspring marked trans-heterozygous (MH) of *Drosophila melanogaster* standard (ST) and high bioactivation capacity (BH) crosses treated with different concentrations of the crude hydroalcoholic bark extract of *A. fraxinifolium*[a]

Genotypes and treatment (mg mL^{-1})	No. of flies (n)	Small single spots (1-2 cells)[b]	Large single spots (>2 cells)[b]	Twin spots (mwh/flr^3)	Total spots	Total spots (mwh[c])
ST cross						
Saline	30	0.57 (17)	0.03 (01)	0.07 (02)	0.67 (20)	18
50	30	0.93 (28)i	0.07 (02)i	0.40 (12)+	1.40 (42)+	42
100	29	1.55 (45)+	0.07 (02)i	1.03 (30)+	2.66 (77)+	77
Urethane (10 mM)	30	2.10 (63)+	0.23 (07)+	0.13 (04)i	2.47 (74)+	72
HB cross						
Saline	30	0.57 (17)	0.00 (00)	0.03 (01)	0.60 (18)	18
50	20	4.20 (84)+	0.40 (08)+	2.00 (40)+	6.60 (132)+	132
100	20	1.95 (39)+	0.35 (07)+	0.95 (19)+	3.25 (65)+	65
Urethane (10 mM)	30	7.23 (217)+	1.93 (58)+	1.03 (31)+	10.20 (306)+	301[a]

Statistical Diagnostics according to Frei and Wurgler (1988), +: Positive, -: Negative, i inconclusive. m: Multiplication factor for the evaluation of results significantly negative, Levels of significance α = β = 0.05, [b]Including single spots flr^3 rares, [c]Considering mwh clones for mwh single spots and twin spots

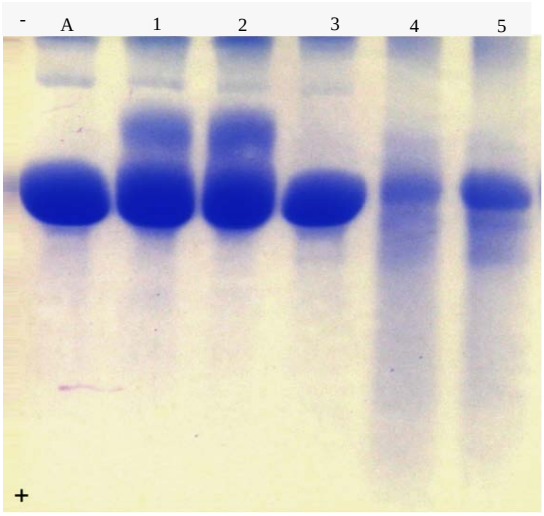

Fig. 1: Effect of ethanolic and hydroethanolic extracts from *Astronium fraxinifolium* and Cu^{2+} on migration of BSA with PAGE (incubation period was 10 days). A: Native BSA, 1: BSA with hydroethanolic extract, 2: BSA with ethanolic extract, 3: BSA with Cu^{2+}, 4: BSA with Cu^{2+} and hydroethanolic extract and 5: BSA with Cu^{2+} and ethanolic extract

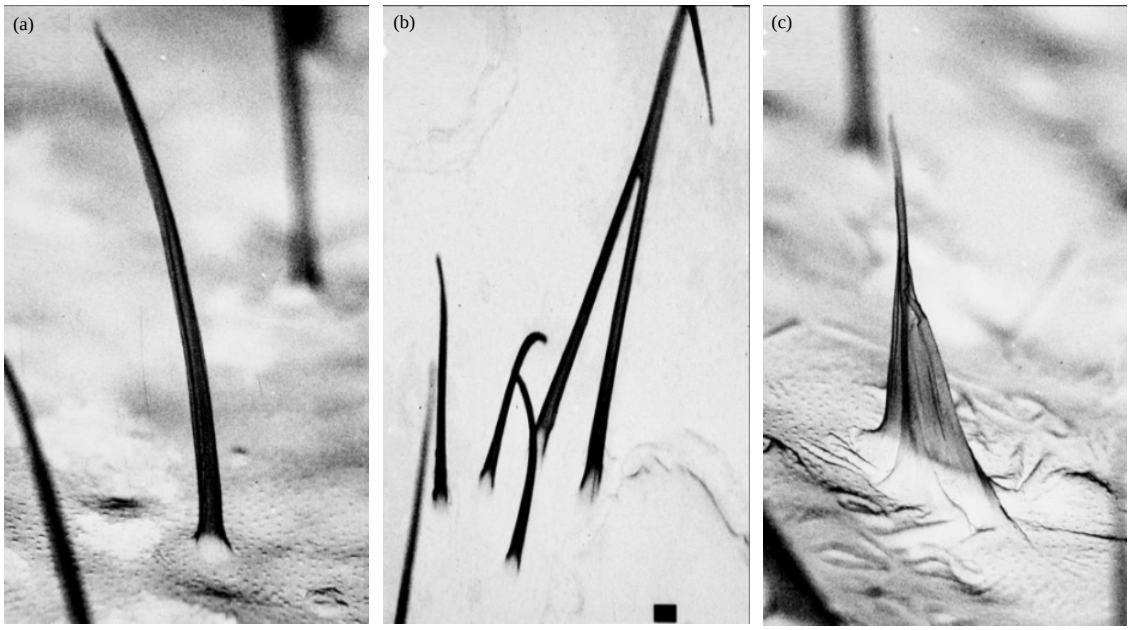

Fig. 2(a-c): Electronic microscopy of mutations present in the wing of *D. melanogaster*, (a): Wild, (b) Multiple wing hair (mwh) mutation and (C): Flare (flr3) mutation (Graf *et al*., 1984, 1989)

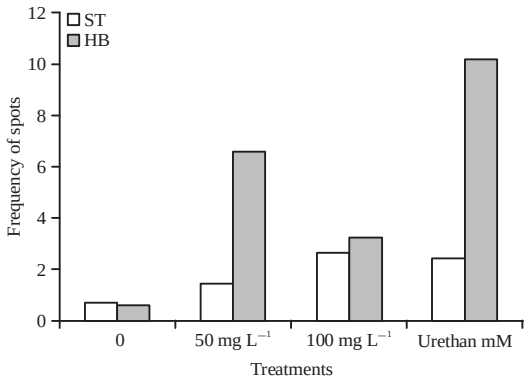

Fig. 3: Mutant spots frequency for ST and HB crosses in different treatments

concentration of 100 mg mL^{-1} (33), a decrease in the amount of chromosomal aberrations is observed compared to other concentrations of crude hydroalcoholic bark extract. Regarding the amount of MN, there was no significant presence of such alteration.

The data relating to the genotoxicity and mutagenicity test of *A. cepa* are shown in Table 4 and Fig. 4.

DISCUSSION

Regarding the action of the extract exclusively on BSA, without copper, there is a possibility that the *A. fraxinifolium* extracts were reacting causing oxidation on BSA, leaving the protein more positivelly charged, when compared to native but without major changes and without breaking peptide bonds. Due to that the space race of the BSA, which suffered action of the extracts was lower than the native BSA.

Already in the presence of extracts and Cu^{2+}, there was an intense action of the extracts together with copper fragmenting the protein. This was the most prominent action in the gel, indicating that the extracts tested have intense oxidative action, it is seen that if the race compared copper together with BSA, which is perceived little change (Fig. 4). Kato *et al*. (1992) showed that metal-catalyzed oxidation of protein can occurs, it depends of the presence of a free radical acting together, such as H_2O_2. In this study, the extracts fulfilled this role. In another study Amici *et al*. (1989) found that there is selective damage of His, Lys, Arg, and Pro in protein and polypeptides by metal-catalyzed oxidation.

According to Graf *et al*. (1996) and Spano *et al*. (2001), the Somatic Mutation And Recombination Test (SMART) in *D. melanogaster* wings proved to be an effective short-term bioassay for the detection of genotoxic or anti-genotoxic activity of pure or complex compounds. Being able to enzymatically activate procarcinogens and promutagens and quantitatively determine the potential for recombination of genotoxic agents analyzed. In the same way as Graf and Singer (1992) and Graf and van Schaik (1992) demonstrated that the HB cross consists in enzymatic increases of CYP450, a

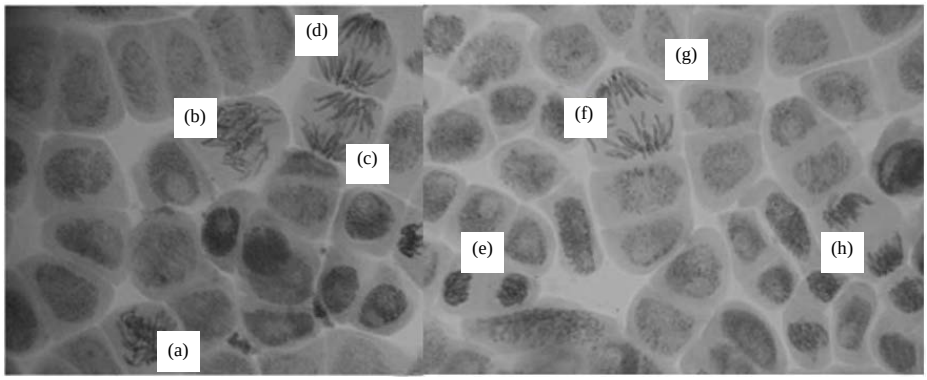

Fig. 4: Chromosomal aberrations found in somatic cells of *A. cepa* root in optical microscope at 100x magnification. Representation of somatic cells of *A. cepa* root in cell division and mutations caused by exposure to crude hydroalcoholic extract of stems/bark of *A. fraxinifolium* (a) aberrant metaphase, not organized chromosomes the equator, (b) aberrant metaphase, not organized chromosomes the equator, (c) aberrant anaphase, chromosomal nondisjunction, (d) normal anaphase, (e) normal telophase, (f) aberrant anaphase, chromosome breaks, (g) micronucleus and (h) aberrant telophase, chromosome non disjunction

Table 4: Mitotic indices and chromosomal aberrations for crude hydroalcoholic bark extract of *A. fraxinifolium* treated at concentrations of 1.0, 10, 50 and 100 mg mL^{-1}

		Aberrations			
Treatments (mg mL^{-1})	Mitotic index (%)	Anaphase	Telophase	Total aberrant cells	Aberrant cells (%)
0	6.82±14.43	1	2	03	0.06±0.05[a]
1	6.44±10.56	70	15	85	1.70±0.35[b]
10	5.72±11.56	50	11	61	1.22±0.25[b]
50	5.40±11.97	45	8	53	1.06±0.36[b]
100	2.92±6.16	30	3	33	0.66±0.15[b]
MMS (10 mg L^{-1})	5.40±11.83	36	14	50	1.00±0.53[b]

Negative control treated with distilled water and positive control treated with Methyl Methane Sulfonate (MMS) 10 mg L^{-1}. About 5000 cells analyzed. Averages± standard deviation. Same letters in columns do not differ statistically, averages evaluated with analysis of variance (ANOVA) ($p \leq 0.05$)

superfamily of enzymes, which are responsible for metabolizing and activating procarcinogens and promutagens.

On the other hand, studies of Levan (1938), Fiskesjo (1985) and Chauhan and Gupta (2005) found efficiency of the *A. cepa* test to indicate chromosomes damage and disorders of the mitotic cycle, due to its sensitivity and good correlation with mammals tests, and its proliferation kinetics characteristics and large chromosomes. Since Fiskesjo (1985) and Leme and Marin-Morales (2009) concluded that the test can be used in genotoxicity and cytotoxicity studies of wide variety of environmental pollutants such as heavy metals, pesticides, aromatics and complex mixtures of pollutants. Besides environmental monitoring testing genotoxicity with *A. cepa* is being applied in the analysis of natural chemical compounds, mainly to check the safety and genotoxicity of plant extracts used in folk medicine as work performed by Camparoto *et al*. (2002), Knoll *et al*. (2006), De Rezende *et al*. (2011), Neves *et al*. (2014) and Cardoso *et al*. (2014).

Bishop and Schiestl (2002, 2003) reported that genotoxic events can lead to homologous recombination that may result in loss of heterozygosity or genetic rearrangements. Some of these genetic alterations are co-related with the manifestation of recessive hereditary diseases and may play a primary role in carcinogenesis. However, they are probably involved in secondary and subsequent steps of carcinogenesis revealing recessive oncogenic mutations.

Ferguson and de Flora (2005) reported that the increasing evidences suggests that cancer and other mutation-related diseases can be prevented not only by limiting exposure to known risk factors but also the amount of protective factors and the modulation of defense of the host organism. This strategy relates to the chemoprevention and may be by pharmacological agents and/or dietary factors, since genotoxic actions or xenobiotics damage are removed by means of various intra and extra cellular mechanisms.

The low number of individuals analyzed in the SMART test Table 3 is due to cytotoxicity of the extract. It was noticed

that there was a significant increase in the number of single spots and total number of spots for both crossings, with the exception of the dose 100 mg mL^{-1} of HB cross, because this concentration were the individuals died before expressing phenotypically spots on the wings. Thus, the test of *A. cepa* Table 4 helped to confirm results obtained in the SMART test, since this demonstrates the increase of chromosomal aberrations due to the action of the hydroalcoholic bark extract of *A. fraxinifolium.*

An increase in the number of single and total spots in the HB cross concentration of 50 mg mL^{-1}, also indicating that the compounds of the hydroalcoholic bark extract of *A. fraxinifolium* need to be metabolized by CYP450 enzyme complex to demonstrate genotoxic action (Table 3).

The decrease in the total frequency of mutant spots at HB cross concentration of 100 mg mL^{-1} in the SMART test and the decrease in mitotic index and the total number of chromosomal aberrations in the concentration of 100 mg mL^{-1} in the *A. cepa* test indicates that the hydroalcoholic bark extract of the *A. fraxinifolium* has cytotoxic activity. Thus, components present in the extract generated cellular damage leading to cell death, preventing to occur their proliferation.

According to Leite (2002), *A. fraxinifolium* features lots of tannin in its bark. Phytochemical screening performed by TLC with the extract of *A. fraxinifolium* corroborates the data presented. Furthermore, tests assessing the amount of total phenols indicate the presence of these compounds (Table 1 and 2).

Moreover, the cytotoxic and genotoxic results presented within the methodologies used, demonstrate that this activity is most likely due to the activity of phenolic compounds present in the extract, mainly tannins.

Tannins are polyphenolic bioflavonoids from secondary metabolism found in fruits and vegetables, which have gained interest because of its widespread beneficial effect on human health (Dixon *et al*., 2005). However, it is known that the astringent properties of tannins provide enzyme inhibition and substrate deprivation. Another property of tannins involves deprivation of metal ions into cells. Finally, a third property of tannins is their action on the cell membrane. (Scalbert, 1991).

CONCLUSION

Genotoxic and cytotoxic actions of hydroethanolic bark extract of *A. fraxinifolium* can be explained by the actions of tannins present in its composition. But there may be other substances that also act for such results. Another possible cell damage caused by this extracts, it is by oxidation, how was presented by in pro-oxidant test, where occurred the fragmentation of BSA, metal catalyzed. Therefore, the species *A. fraxinifolium* needs further study about its components and therapeutic actions.

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